


Genes for wheat stem rust resistance postulated in German cultivars and their efficacy in seedling and adult-plant field tests

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Abstract

Stem rust of wheat (caused by *Puccinia graminis* f.sp. *tritici*) gained high international attention in the last two decades, but does not occur regularly in Germany. Motivated by a regional epidemic in 2013, we analysed 15 spring and 82 winter wheat cultivars registered in Germany for their resistance to stem rust at the seedling stage and tested 79 of these winter wheat cultivars at the adult-plant stage. A total of five seedling stem rust resistance genes were postulated: *Sr38* occurred most frequently ($n = 29$), followed by *Sr31* ($n = 11$) and *Sr24* ($n = 8$). *Sr7a* and *Sr8a* occurred only in two spring wheat genotypes each. Four cultivars had effective seedling resistance to all races evaluated that could only be explained by postulating additional resistance genes ('Hyland', 'Pilgrim PZO', 'Tybalt') or unidentified gene(s) ('Memory'). The three winter wheat cultivars ('Hyland' 'Memory' and 'Pilgrim PZO') were also highly resistant at the adult-plant stage; 'Tybalt' was not tested. Resistance genes *Sr24* and *Sr31* highly protected winter wheat cultivars from stem rust at the adult-plant stage in the field. Disease responses of cultivars carrying *Sr38* varied. Mean field stem rust severity of cultivars without postulated seedling resistance genes ranged from 2.71% to 41.51%, nine of which were significantly less diseased than the most susceptible cultivar. This suggests adult-plant resistance to stem rust may be present in German wheat cultivars.

KEYWORDS

cultivars, *Puccinia graminis* f. sp. *tritici*, resistance, wheat stem rust

1 | INTRODUCTION

Wheat stem rust, caused by *Puccinia graminis* f. sp. *tritici* (Pgt), has gained international attention since the occurrence of race TTKSK (Ug99) that was able to infect wheat (*Triticum aestivum* L.) varieties with stem rust resistance gene *Sr31* (Singh et al., 2015). This gene, originally derived from wheat x rye (*Secale cereale* L.) hybrid derivatives produced in Germany in the 1930s (Mettin, Bluthner, & Schlegel, 1973; Zeller, 1973), was widely distributed by the CIMMYT wheat breeding programme (e.g., Bobwhite and Veery selections) and continues to occur at

high frequencies in CIMMYT breeding populations (<http://www.globalrust.org/gene/Sr31>). *Sr31* protected wheat globally from being infected by stem rust for decades. Since first reported in Uganda (Pretorius, Singh, Wagoire, & Payne, 2000), the Ug99 race group rapidly expanded its geographical range in East Africa and the Middle East and reached the Nile delta of Egypt in 2014 (Patpour et al., 2016; Singh et al., 2015). Thus, a jump across the Mediterranean Sea into Greek or Italian wheat-growing areas would be possible in future.

In European countries, stem rust is of variable importance. Its occurrence and the magnitude of damage depend largely upon

climatic conditions, virulence of the pathogen population and host genotypes. Areas with a continental climate with short and hot summers are regularly threatened by wheat stem rust, such as Russia (Skolotneva, Maleeva, Insarova, & Lekomtseva, 2008), where virulent races are frequent in epidemic years (Lekomtseva, Volkova, Zaitseva, Skolotneva, & Chaika, 2007), Kazakhstan (Shamanin et al., 2016) and Turkey (Mert, Karakaya, Düşünceli, Akan, & Çetin, 2012). In North and Central Europe, the most severe stem rust epidemics occurred in the first half of the twentieth century. National wheat yield losses of 9–33% occurred in Scandinavia in 1951, and losses of 5–20% were reported in Eastern and Central Europe in 1932 (Zadoks, 1963). Similarly, in Austria, the last severe epidemics occurred decades ago, in 1972 and 1977 (Zwatz, 1982). In Germany, wheat stem rust is at present not an important disease although rye stem rust (caused by *P. graminis* f. sp. *secale*; *Pgs*) occurs frequently (Miedaner et al., 2016).

Wheat stem rust reoccurred in Germany in 2013, when a cold and wet spring delayed winter wheat development for about 2 weeks and unusually high temperatures, starting at the beginning of June, favoured disease development (Flath, Sommerfeldt-Impe, & Schmitt, 2014; Olivera Firpo et al., 2017). From this regionally restricted epidemic, six races with different percentages of occurrence were identified among 48 German *Pgt* isolates: TKTF (33% occurrence), TKKF (40%), TKPF (4%), TKKP (10%), PKPF (6%) and MMMTF (6%) with none of these races belonging to the TTKS (Ug99) race group (Olivera Firpo et al., 2017). In 2016, wheat stem rust was found again in spring wheat in Germany (Olivera Firpo et al., 2017). In the same year, stem rust was reported on durum wheat in Sicily (Patpour & Hovmøller, 2016).

In addition to the potential aerial incursion of new races from neighbouring wheat-producing regions, the presence of the alternate host of the stem rust pathogen near wheat fields has the potential of increasing the occurrence of new virulence combinations. Common barberry (*Berberis vulgaris* L.), the most important alternate host of the wheat stem rust pathogen, is native to many German regions (<http://www.floraweb.de/webkarten/karte.html?taxnr=818>) despite large eradication efforts starting with a regional legislative decree in 1805 (Lehmann, Kummer, & Dannenmann, 1937) and being continued through at least 1962 (Anonymous, 1954, Hinke, 1955). In addition to being a source of new virulence combinations, the alternate host of the stem rust pathogen is a source of early and locally produced inoculum (aeciospores) (Roelfs, Singh, & Saari, 1992). The main causes for the infrequent occurrence of wheat stem rust in Germany might be a higher temperature optimum for *Pgt* infection compared to *Pgs* and/or lack of adequate time between the release of aeciospores from barberry and the more rapidly maturing winter wheat crop. In addition, winter wheat is more common than spring wheat in Germany.

Potential sources of stem rust resistance have been identified in several studies with European wheat cultivars, and the presence of at least 17 seedling resistance genes has been postulated to date (Pathan & Park, 2007; Randhawa, Bansal, Lillemo, Miah, & Bariana, 2016; Singh, Park, McIntosh, & Bariana, 2008). However, stem rust

resistance in recent German cultivars has not been investigated. In the official Austrian variety trials, all wheat cultivars were assayed for response to *Pgt* since 1990 (Oberforster, Plank, & Bedlan, 2010). The disease could be induced every year with mean severities varying from 5% to 59% in the period from 1999 to 2009. The 40–52 wheat cultivars tested each year significantly varied in their stem rust severity with a range from 0% to 100%. Reduction of thousand-grain weight ranged from 0% to 46% among the 51 wheat cultivars tested in an artificial epidemic in 2004 (Oberforster et al., 2010). This result demonstrates the potential yield damage incurred by stem rust that should be a motivation for increasing the research on this host–pathogen interaction. Moreover, with the re-emergence of common barberry in the wake of discontinued eradication programmes and global climate change where increased temperatures are predicted, stem rust might reappear as a devastating disease throughout Europe. Temperature increased in Germany one degree more than the linear worldwide trend calculated for the period from 1881 to 2015 (DWD, 2016). Moreover, 23 of the 25 years from 1991 to 2016 have been warmer than the mean value of the reference period of 1961–1990 (DWD, 2016). Breeding for resistance is therefore necessary to combat thermophilic pathogens such as *Pgt*.

Genetic resistance to cereal rusts is inherited by (i) individual race-specific genes that are effective during the whole lifespan of a crop and that can be monitored at the seedling stage (all-stage resistance, McIntosh, Wellings, & Park, 1995) or (ii) adult-plant resistance (APR) inherited by either single or many genes (Miedaner, 2016). Because of the limited information about stem rust resistance genes in modern German wheat cultivars, our objectives were to (i) evaluate resistance in a set of 97 winter and spring wheat cultivars registered in Germany to the available *Pgt* isolates from the 2013 German stem rust epidemic at the seedling stage; (ii) postulate *Sr* resistance genes in these cultivars by seedling inoculation with 13 international *Pgt* isolates; and (iii) assess resistance at the adult-plant stage of the winter wheat cultivars in inoculated field experiments in Germany across 3 years.

2 | MATERIALS AND METHODS

2.1 | Wheat genotypes

This study included 15 spring and 82 winter wheat cultivars tested for stem rust response at the seedling stage. All cultivars are registered in the recent German Descriptive Lists of Cultivars (BSL, 2015, 2016) and were released between 1990 and 2016 and bred by several private plant breeding companies (Tables S1 and S2). In the field, 79 of the winter wheat cultivars were tested.

2.2 | Stem rust evaluation of wheat germplasm

2.2.1 | Seedling evaluations

Stem rust seedling tests were conducted in 2015 and 2016 at the University of Minnesota and the USDA-ARS Cereal Disease

TABLE 1 Race and isolate designation, origin and virulence phenotype of *Puccinia graminis* f. sp. *tritici* isolates used to evaluate seedling resistance in German wheat cultivars

Race	Isolate	Origin	Avirulence/virulence
TTKSK ^a	04KEN156/04	Kenya	Sr24 36 Tmp/Sr5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 30 31 38 McN
TRTTF	06YEM34-1	Yemen	Sr8a 24 31/Sr5 6 7b 9a 9b 9d 9e 9g 10 11 17 21 30 36 38 McN Tmp
TKTTF	13ETH18-1	Ethiopia	Sr11 24 31/Sr5 6 7b 8a 9a 9b 9d 9e 9g 10 17 21 30 36 38 McN Tmp
TKTTF	13GER17-2	Germany	Sr11 24 31/Sr5 6 7b 8a 9a 9b 9d 9e 9g 10 17 21 30 36 38 McN Tmp
TKKTP	13GER16-1	Germany	Sr11 31 36/Sr5 6 7b 8a 9a 9b 9d 9e 9g 10 17 21 24 30 38 McN Tmp
MMMTF	13GER04-1	Germany	Sr6 8a 9b 9e 21 24 30 31/Sr5 7b 9a 9d 9g 10 11 17 36 38 McN Tmp
TTTTF	02MN84A-1-2	USA	Sr24 31/Sr5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 30 36 38 McN Tmp
TPMKC	74MN1409	USA	Sr6 9a 9b 24 30 31 38/Sr5 7b 8a 9d 9e 9g 10 11 17 21 36 McN Tmp
RKRQC	99KS76A-1	USA	Sr9e 10 11 24 30 31 38 Tmp/Sr5 6 7b 8a 9a 9b 9d 9g 17 21 36 McN
QTHJC	75ND717C	USA	Sr7b 9a 9e 24 30 31 36 38 Tmp/Sr5 6 8a 9b 9d 9g 10 11 17 McN
QFCSC	06ND76C	USA	Sr6 7b 9b 9e 11 24 30 31 36 38 Tmp/Sr5 8a 9a 9d 9g 10 17 21 McN
QCCSM	75WA165-2A	USA	Sr6 7b 8a 9b 9e 11 30 31 36 38 Tmp/Sr5 9a 9d 9g 10 17 21 24 McN
MCCFC	59KS19	USA	Sr6 8a 9a 9b 9d 9e 11 21 24 30 31 36 38/Sr5 7b 9g 10 17 McN Tmp

^aRace nomenclature was based on Roelfs and Martens (1988) and Jin et al. (2008).

Laboratory, St. Paul, MN. Thirteen *Pgt* races with different virulence combinations and geographic origins were used to postulate the presence of *Sr* genes in the 97 German cultivars: TTKSK (Kenya), TRTTF (Yemen), TKTTF (Ethiopia), TKTTF (Germany), TKKTP (Germany), MMMTF (Germany), TTTTTF (USA), TPMKC (USA), RKRQC (USA), QTHJC (USA), QCCSM (USA), QFCSC (USA) and MCCFC (USA) (Table 1). The race designations are based on the letter code nomenclature system (Roelfs, Long, & Roberts, 1993; Roelfs & Martens, 1988), modified to further delineate races in the TTKS (Ug99) group (Jin et al., 2008). Screening assays with isolates from outside the United States were conducted inside a University of Minnesota Biosafety Level 3 (BSL-3) facility, whereas the evaluations with US isolates were performed in the USDA-ARS Cereal Disease Laboratory glasshouses. Experimental procedures for storage retrieving, inoculation, incubation and disease assessment were carried out as described by Jin et al. (2007) and Olivera et al. (2015). In short, fully expanded primary leaves of five seedlings per cultivar were inoculated 8–9 days after planting. Wheat cultivar 'McNair 701' (Citr 15288) was used as a susceptible control. All assessments were repeated in separate inoculation experiments on different dates. Seedling infection types were determined at 14 days after inoculation following the 0–4 scale developed by Stakman, Steward, and Loegering (1962). Infection types greater than or equal to three were categorized as susceptible reactions, whereas those less than three were categorized as resistant reactions.

2.2.2 | Adult-plant evaluations in the field

For field stem rust responses, 79 winter wheat cultivars were tested. Entries were arranged together with two check cultivars (Brilliant and Julius) according to a randomized complete block

design with three replicates repeated over 3 years (2014, 2015, 2016) at the trial site of JKI in Berlin-Dahlem (DAL, latitude 52°44'94"N, longitude 13°27'68"E, 45 m above sea level, 600 mm mean annual precipitation, 8.8°C mean annual temperature). Each entry was grown in three-row microplots 0.5 m long and 0.6 m wide. Inoculations were performed with a mixture of five *Pgt* races sampled from the German epidemic in 2013 and characterized at the USDA-ARS Cereal Disease Laboratory (Table 2). Together, they comprised virulences for all *Sr* genes from the international *Pgt* differentials except for *Sr*24 and *Sr*31. Urediniospores were multiplied on seven-day-old seedlings of susceptible wheat cultivars ('McNair', 'Triso') by spraying the primary and secondary leaves with a 0.1% agar-urediniospore suspension. Plants were kept for 24 hr at 21°C and 100% humidity in the dark and for the remainder of time at 21°C, 10,000 Lux and 16-hr photoperiod in a climate chamber with 60% humidity. After 12 days, the new urediniospores were collected, dried for 12 hr on silica gel and stored in a –80°C freezer. Isolates were mixed in equal amounts prior to inoculation. Inoculation was carried out at EC59–65 growing stage (end of heading through the middle of flowering) by applying a suspension of 500 ml of mineral oil (Isopar M) and 100 mg spores per 100 m² by a microsprayer from above (Micron Ulva, Bromyard Industrial Estate, Bromyard, Herefordshire HR7 4HS, UK) directly on the plants of each plot. Stem rust severity was assessed at weekly intervals three times until full ripening stage. The stem segment between the second leaf from top (F-1) and the next internode was visually scored for percentage of the stem covered by rust (0%–100%) on a plotwise basis. Mean disease severity was calculated according to the area under disease progress curve for every plot using the program package RESI2 (Moll, Flath, & Tessenow, 2010). A score was converted from the mean stem rust severity to a logarithmic scale from 1 to 9 with

1 = 0–0.75% and 9 = >60% mean disease severity. For comparison of the cultivars, we used least-squares means (LS-means) that are defined as a linear combination (sum) of the estimated effects (means, etc.) from a linear model. This estimate is considered more appropriate than using normal means because of missing values. The multiple tests were performed with SIMULATE within the program package RESI2 based on SAS (Moll et al., 2010). Edwards and Berry (1987) proposed this procedure, which uses pivotal statistics, for multiple comparisons in linear and mixed models. Basically, this is an extension of the Tukey test that is also appropriate for unbalanced trial designs and accounts for correction of *p* values according to the number of comparisons. The procedure was shown to be preferable to the Bonferroni test (Piepho, 2000).

TABLE 2 Race designation and virulence phenotype of *Puccinia graminis* f. sp. *tritici* isolates of German origin used to evaluate field resistance in German winter wheat cultivars

Race	Avirulence/virulence
TKTTF	Sr11 24 31/Sr5 6 7b 8a 9a 9b 9d 9e 9g 10 17 21 30 36 38 McN Tmp
TKPTF	Sr9b 11 24 31/Sr5 6 7b 8a 9a 9d 9e 9g 10 17 21 30 36 38 McN Tmp
MMMTF	Sr6 8a 9b 9e 21 24 30 31/Sr5 7b 9a 9d 9g 10 11 17 36 38 McN Tmp
MRRTF	Sr8a 9e 21 24 30 31/Sr5 6 7b 9a 9b 9d 9g 10 11 17 36 38 McN Tmp
MRMTF	Sr8a 9b 9e 21 24 30 31/Sr5 6 7b 9a 9d 9g 10 11 17 36 38 McN Tmp

TABLE 3 Number and percentage of German wheat cultivars exhibiting resistant and susceptible seedling reactions to thirteen isolates of *Puccinia graminis* f. sp. *tritici*

Race	Origin	Resistant		Susceptible	
		No.	%	No.	%
TTKSK	Kenya	11	11.3	86	88.7
TRTTF	Yemen	24	24.7	73	75.3
TKTTF	Ethiopia	28	28.9	69	71.1
TKTTF	Germany	22	22.7	75	77.3
TKKTP	Germany	14	14.4	83	85.6
MMMTF	Germany	23	23.7	74	76.3
TTTTF	USA	27	27.8	70	72.1
TPMKC	USA	55	56.7	42	43.3
RKQQC	USA	56	57.7	41	42.3
QTHJC	USA	54	55.7	43	44.3
QFCSC	USA	57	58.8	40	41.2
QCCSM	USA	62	63.9	35	36.1
MCCFC	USA	55	56.7	42	43.3
All Races		4	4.1	27	28.1

2.3 | Molecular marker analysis

Genomic DNA was isolated from 10-day-old seedlings following a modified cetyltrimethylammonium bromide extraction method (Rouse, Nava, Chao, Anderson, & Jin, 2012). The amount and purity of DNA was determined using a NanoDrop ND-1000 (NanoDrop Products). Molecular markers specific to seedling resistance genes *Sr8a* (Hiebert, Rouse, Nirmala, & Fetch, 2017), *Sr24* (Mago et al., 2005), *Sr31* (Mohler, Hsam, Zeller, & Wenzel, 2001) and *Sr38* (Helguera et al., 2003) were assessed to confirm the presence or absence of these genes that had been postulated in seedling tests. In addition, we assessed molecular markers linked to APR gene *Sr2* (Mago et al., 2011) and to the pleiotropic APR genes *Sr55/Lr67/Yr46/Pm46* (Forrest et al., 2014), *Sr57/Lr34/Yr18/Pm38* (Lagudah et al., 2009) and *Sr58/Lr46/Yr29/Pm39* (www.integratedbreeding.net) to postulate the presence of these genes.

3 | RESULTS

The seedling test was performed with 13 *Pgt* isolates selected for a maximal diversity of virulence combinations and for their international importance including three races that cover most of the virulences detected in Germany (Table S3). Seedling resistance was observed frequently among the 97 German cultivars (Table 3). Even to race TTKSK (Ug99), 11 cultivars showed resistance. To the three German races TKTTF, TKKTP and MMMTF isolated from the 2013 epidemic, 14–23 cultivars were resistant. To the seven US *Pgt* races, German wheat cultivars provided a reservoir of seedling resistances with frequencies ranging from 28% to 64%. This was valid even for the most virulent race (TTTTF) for which 27 resistant cultivars were detected.

Five stem rust resistance genes were postulated by seedling reaction patterns in 53 of the 97 wheat cultivars tested, with *Sr38* occurring most frequently ($n = 29$), followed by *Sr31* ($n = 9$) and *Sr24* ($n = 7$; Tables 4 and S3). These genes were postulated only in winter cultivars, except for *Sr24* that was also postulated to be present in the spring wheat cultivar 'Quintus'. Seedling resistance genes *Sr7a* and *Sr8a* were found in only two spring wheat cultivars each. Two spring ('Tybalt' and 'Taifun') and three winter cultivars ('Hyland', 'Memory' and 'Pilgrim PZO') contain more than one stem rust resistance gene (Table 5).

We applied DNA markers to confirm the presence of four of the five genes postulated to be present in this set of German cultivars. Resistance genes *Sr8a*, *Sr24* and *Sr31* were confirmed in all the lines postulated to carry these genes (Table 4). However, for *Sr38*, the DNA marker test was negative in one cultivar ('Bernstein') that we postulated to possess *Sr38* based on seedling *Pgt* phenotypes. The four cultivars that were resistant to all races were assessed with the *Sr24* and *Sr31* markers. Cultivar 'Memory' tested negative for both markers, indicating the absence of both resistance genes (Table 5). Cultivars 'Hyland' and 'Pilgrim PZO' carry *Sr31*, whereas cultivar 'Tybalt' carries *Sr24*. These four cultivars and spring wheat 'Taifun'

TABLE 4 Gene postulation of German wheat cultivars in response to 13 different races of *Puccinia graminis* f.sp. *tritici* (*Pgt*); DNA-based markers confirmed (+) or rejected (–) postulated *Sr* genes (for infection types to the *Pgt* races, please refer to Table S3)

Cultivar	Gene postulation	Marker confirmation
Granus (S)	<i>Sr7a</i>	n.t.
Lennox (S)	<i>Sr7a</i>	n.t.
Taifun (S)	<i>Sr8a</i> [±] ^a	+
KWS Scirocco (S)	<i>Sr8a</i>	+
Quintus (S)	<i>Sr24</i>	+
Elixer	<i>Sr24</i>	+
Gordian	<i>Sr24</i>	+
Desamo	<i>Sr24</i>	+
Dichter	<i>Sr24</i>	+
KWS Magic	<i>Sr24</i>	+
KWS Loft	<i>Sr24</i>	+
Winnetou	<i>Sr31</i>	+
Brilliant	<i>Sr31</i>	+
Pamier	<i>Sr31</i>	+
Joker	<i>Sr31</i>	+
Forum	<i>Sr31</i>	+
Rebell	<i>Sr31</i>	+
Anapolis	<i>Sr31</i>	+
Sarmund	<i>Sr31</i>	+
Alfons	<i>Sr31</i>	+
Tommi	<i>Sr38</i>	+
Impression	<i>Sr38</i>	+
Manager	<i>Sr38</i>	+
Potenzial	<i>Sr38</i>	+
Mulan	<i>Sr38</i>	+
Tabasco	<i>Sr38</i>	+
Arktis	<i>Sr38</i>	+
Genius	<i>Sr38</i>	+
Linus	<i>Sr38</i>	+
Meister	<i>Sr38</i>	+
Colonia	<i>Sr38</i>	+
Opal	<i>Sr38</i>	+
Bombus	<i>Sr38</i>	+
Atomic	<i>Sr38</i>	+
Capone	<i>Sr38</i>	+
Pionier	<i>Sr38</i>	+
Avenir	<i>Sr38</i>	+
Mescal	<i>Sr38</i>	+
Landsknecht	<i>Sr38</i>	+
KWS Milaneco	<i>Sr38</i>	+
Kompass	<i>Sr38</i>	+
RGT Reform	<i>Sr38</i>	+
KWS Montana	<i>Sr38</i>	+

(Continues)

TABLE 4 (Continued)

Cultivar	Gene postulation	Marker confirmation
Spontan	<i>Sr38</i>	+
Axioma	<i>Sr38</i>	+
Franz	<i>Sr38</i>	+
Bernstein	<i>Sr38</i>	–
Kobold	<i>Sr38</i>	+
Ohio	<i>Sr38</i>	+

S, spring wheat cultivar; n.t., not tested.

^aAdditional seedling resistance gene present.

carry additional resistance gene(s) that could not be postulated with the races and markers used in this study.

German cultivars with postulated seedling *Sr* genes were more resistant in field evaluations, with scores ranging from 1 to 6, compared to those without postulated *Sr* genes ranging from 3 to 8 (Figure 1). Despite this, a few German cultivars without postulated *Sr* genes had relatively low stem rust scores from 3 to 4. Interestingly, genotypes without postulated *Sr* genes showed a normal distribution, whereas those with *Sr* genes did not. Both *Sr24* and *Sr31* were highly effective at the adult-plant stage in the field showing a similar low mean stem rust severity (Table 6). None of the cultivars with one of these genes had a score higher than 4. For *Sr38*, considerable variation in adult-plant stage was observed in mean stem rust severity ranging from score 1 to 6. The three winter wheat cultivars with additional unidentified seedling resistance gene(s) and tested at the adult-plant stage were strongly resistant (score 1–2). This might have been due to *Sr31* in two cases. Thirty-four winter wheat cultivars susceptible to all races evaluated at the seedling stage exhibited a disease severity in field evaluations that ranged from scores 3 to 8 (Table 7). Nine of these cultivars were significantly ($p < .01$) less diseased than the most susceptible cultivar 'Julius' as shown by a multiple statistical test. Number of genotypes and their stem rust response classification from seedling and field tests are summarized in Table S4.

Additionally, we analysed the 79 field-tested winter wheat cultivars with molecular markers linked to four APR genes. No cultivar was found to possess the marker haplotypes associated with *Sr2* or *Sr55/Lr67/Yr46/Pm46*. For markers linked to *Sr57/Lr34/Yr18/Pm38*, one cultivar (Waxydie) possessed the resistance-linked haplotype. Forty-one cultivars showed the marker allele associated with *Sr58/Lr46/Yr29/Pm39*.

4 | DISCUSSION

This study reports the seedling and adult-plant stem rust resistance response in recent, commercially grown German wheat cultivars. The lack of information and effort in characterizing the basis of stem rust resistance in German breeding programmes is because of the rare natural occurrence of stem rust in this country. We analysed 15

TABLE 5 Seedling infection types of German wheat cultivars with unidentified seedling resistance in response to different races of *Puccinia graminis* f. sp. *tritici*; DNA-based markers confirmed (+) or rejected (–) postulated *Sr* genes

Cultivar	TTKSK ^a 04KEN156/04	TRTTF 06YEM34-1	TKTTF 13ETH18-1	TKTTF 13GER17-2	TKKTP 13GER16-1	MMMTF 13GER04-1	TTTTF 01MN84A-1-2	TPMKC 74MN1409	RKQCC 99K576A-A	QTHJC 75ND717C	QCCSM 75WA165-2A	QFCSC 06ND76C	MCCFC 59KS19	Gene postulation	Marker confirmation	
															Sr24	Sr31
Hyland	2– ^b	2–	2–	2–	2–	2–;	2–	2–	2–	2–	2–	2–	2–	Sr31 + ^c	–	+
Memory	2–	2–	:2–	2–;	2	2–;	2–;	:2–	:1–	:1–	:1–	;	;	U ^d	–	–
Tybal (S) ^e	2–	2–;	0;	2–	2	0;	:1–	2–	2–;	2–	2–	:2–	2–	Sr24 +	+	–
Pilgrim PZO	2–	2–	2–;	2	2+	2–;	2–	2–	2–	2–	2–	2–	2–	Sr31 +	–	+

^aRace nomenclature was based on Roelfs and Martens (1988) and Jin et al. (2008).

^bInfection types (ITs) observed on seedlings at 14 days postinoculation using a 0–4 scale according to Stakman et al. (1962), where ITs of 0, 1, 2, or combinations thereof are considered as a low IT and ITs of three or higher are considered as a high IT.

^cAdditional seedling resistance(s) gene present.

^dU = unknown seedling resistance gene(s).

^eS = spring wheat cultivar.

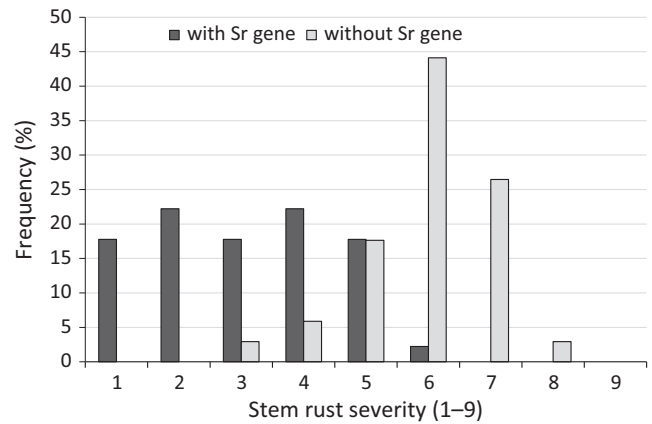


FIGURE 1 Frequency distribution of adult-plant stem rust severity in 79 commercial German winter wheat cultivars with and without postulated *Sr* genes after inoculation with *Pgt* in the field in 3 years

spring and 82 winter wheat cultivars at the seedling stage with a panel of 13 *Pgt* isolates with known virulences, including race TTKSK (Ug99, Table S3). Seventy-nine of the winter wheats were also tested in field adult-plant trials across 3 years.

4.1 | Seedling resistance

Results from this study demonstrate that stem rust resistance is present in German cultivars against race TTKSK and other *Pgt* races of different origins representing a broad virulence spectrum. Of 97 German wheat cultivars evaluated, 54.6% had postulated seedling resistance genes, including *Sr38*, *Sr31*, *Sr24*, *Sr7a* and *Sr8a* although German breeders have not selected for stem rust resistance in the last several decades. The genes must have been introgressed unintentionally into the German germplasm using *Sr* donors for improving other traits. Accordingly, in the Swiss winter wheat cultivar ‘Arina,’ the seedling resistance gene *Sr48* and adult-plant resistance gene *Sr56* have been detected although this cultivar has not knowingly been selected for resistance to stem rust (Bansal et al., 2009, 2014). Our results are in agreement with previous gene postulation studies conducted on European cultivars where *Sr38*, *Sr31*, *Sr24* and *Sr8a* were also identified (Pathan & Park, 2007; Randhawa et al., 2016; Singh et al., 2008). Cultivars with *Sr38* and *Sr31* were most common. This matches results from both hard and soft US winter wheat germplasm where *Sr31* and *Sr38* were also the prevalent *Sr* resistance genes (Zhang, Bowden, Yu, Carver, & Bai, 2014).

Sr38, a gene derived from the wild wheat relative species *Aegilops ventricosa* Tausch, was found to be the most prevalent stem rust resistance gene in German winter wheat. Introgression of the resistance gene *Pch1* effective against eyespot or strawbreaker (caused by *Oculimacula yallundae*) from donor lines VPM1 and the French cultivars ‘Renan’ and ‘Rendezvous’ (Bariana, 1991) presumably led to *Sr38* being so frequent in German cultivars although the two resistance genes are located on different chromosomes. Additionally, *Sr38* is completely linked with resistance to leaf rust (*Lr37*) and stripe

TABLE 6 Adult-plant stem rust severity (LS-means) in field evaluations in 2014–2016 for winter wheat varieties with postulated Sr genes

Cultivar	Gene postulated	Adult-plant severity				
		2014 (%)	2015 (%)	2016 (%)	Mean (%)	Mean score (1–9) ^a
Dichter	Sr24	—	1.07	1.13	0.00	1
KWS Magic	Sr24	—	0.15	0.00	0.00	1
KWS Loft	Sr24	—	0.74	1.96	0.00	1
Elixer	Sr24	—	1.78	2.30	0.04	1
Desamo	Sr24	2.36	0.00	0.00	1.27	2
Gordian	Sr24	4.31	0.14	—	3.72	3
Mean Sr24^b		3.34	0.65	1.08	0.84	1
Alfons	Sr31	—	0.00	0.34	0.00	1
Brilliant	Sr31	0.55	0.13	0.00	0.61	1
Anapolis	Sr31	0.46	0.75	0.78	0.99	2
Pamier	Sr31	0.00	0.00	2.45	1.09	2
Rebell	Sr31	1.26	0.82	0.00	1.09	2
Joker	Sr31	0.00	1.07	—	1.71	2
Sarmund	Sr31	—	0.00	7.49	1.72	2
Winnetou	Sr31	0.69	1.21	—	2.16	3
Forum	Sr31	4.78	0.58	—	4.18	4
Mean Sr31		1.11	0.51	1.84	1.51	2
Spontan	Sr38	—	3.15	1.94	0.55	1
Arktis	Sr38	—	3.61	—	1.33	2
KWS Montana	Sr38	—	7.25	0.00	1.65	2
Opal	Sr38	1.61	3.75	0.00	2.12	3
Linus	Sr38	2.13	4.02	0.00	2.41	3
Ohio	Sr38	—	7.40	2.13	2.78	3
Potenzial	Sr38	1.02	3.79	3.73	3.04	3
Kompass	Sr38	—	6.58	3.94	3.27	3
Impression	Sr38	4.09	4.82	0.00	3.42	3
Tabasco	Sr38	2.90	7.95	0.00	3.92	4
Mescal	Sr38	3.94	5.94	3.45	4.76	4
Avenir	Sr38	7.67	5.41	0.19	5.07	4
Capone	Sr38	2.18	6.08	—	5.13	4
KWS Milaneco	Sr38	7.49	6.57	0.00	5.30	4
Axioma	Sr38	—	8.60	6.91	5.76	4
Bombus	Sr38	5.10	4.53	—	6.09	4
Colonia	Sr38	—	—	7.92	6.20	4
Meister	Sr38	7.64	6.84	5.08	6.97	4
Bernstein	Sr38	—	10.49	9.24	7.87	5
Kobold	Sr38	—	15.16	5.92	8.58	5
Mulan	Sr38	13.26	4.19	5.97	8.63	5
Franz	Sr38	—	11.84	10.48	9.16	5
Atomic	Sr38	11.36	15.31	1.02	9.80	5
Manager	Sr38	5.56	12.86	—	10.01	5
Genius	Sr38	—	15.23	8.79	10.03	5
Tommi	Sr38	3.14	20.50	—	12.00	5
Landsknecht	Sr38	7.74	27.25	15.02	16.26	6
Mean Sr38		5.43	8.81	4.17	6.00	4

(Continues)

TABLE 6 (Continued)

Cultivar	Gene postulated	Adult-plant severity				
		2014 (%)	2015 (%)	2016 (%)	Mean (%)	Mean score (1–9) ^a
Pilgrim PZO	<i>Sr31</i> + ^c	—	3.24	0.00	0.00	1
Hyland	<i>Sr31</i> +	0.00	1.48	1.19	1.16	2
Memory	U ^d	0.00	1.16	1.14	1.05	2
Mean		0.00	1.96	0.78	0.74	2

^aScore 1–3 = resistant (R), 4–6 = moderately resistant (MR) to moderately susceptible (MS), 7–9 = susceptible (S).

^bMeans of varieties with the same *Sr* gene are printed in bold.

^cAdditional seedling resistance(s) gene present.

^dU = unknown seedling resistance gene(s) (see Table 5).

rust (*Yr17*) (Bariana & McIntosh, 1993). The *Yr17* resistance widely introduced into European cultivars that was effective through the late 1990s in Germany might be another cause for the introgression of *Sr38*, because stripe rust (caused by *P. striiformis* f. sp. *tritici*; *Pst*) is an important wheat disease in Germany. Indeed, all but three *Sr38* carriers in our study were superior for yellow rust resistance with scores from 1 to 4 on the 1–9 scale (BSL, 2015, 2016).

Sr31, originally introduced from rye, provided strong resistance to all German *Pgt* races identified in the 2013 local epidemic (Olivera Firpo et al., 2017). The gene was most probably introduced in German wheats by the widespread use of wheat–rye translocations that provided very high yield potential and originally resistance to powdery mildew and several rusts (Bartos, 1993). In particular, the linkage with the resistance gene *Yr9* effective against stripe rust might have been advantageous before the breakdown of this gene by virulent *Pst* races. In an earlier study on 105 European wheats from nine countries including Germany, *Sr31* was the most frequent stem rust resistance gene (18%, Pathan & Park, 2007), was widely distributed in Hungarian wheat (Purnhauser, Bóna, & Láng, 2011) and was postulated in wheat cultivars from the UK (Singh et al., 2008). *Sr31* was, however, defeated by the TTKSK race group (Table S3), and it is questionable whether this globally distributed gene should be further used, although this race group has not appeared in Europe to date.

Sr24, originally derived from *Agropyron elongatum* (Host) P.Beauv. (*syn. Thinopyrum ponticum*, <http://www.globalrust.org/gene/sr24>), is linked to *Lr24*. It is present in relatively high frequencies in Australian, South and North American, and South African wheats (Jin et al., 2008). *Sr24* confers resistance to most *Pgt* races in the world including many variants of the TTKSK race group (Jin et al., 2007). However, virulence to *Sr24* has already been identified in South Africa (Le Roux & Rijkenberg, 1987), North America (Olivera, Pretorius, Badebo, & Jin, 2012) and India (Bhardwaj et al., 1990). In addition, the variants PTKST, TTKST and TTKTT of the Ug99 race group include virulence to *Sr24* (Newcomb et al., 2016). Virulence to *Sr24* was also observed in race TKKTP, which was isolated from samples collected in the 2013 stem rust epidemic in Germany (Olivera Firpo et al., 2017). Although present at a low frequency of 10%, race TKKTP is of special concern due to its unique combined virulences to *Sr24*, *SrTmp* and the temporary designated resistance gene *Sr1RS*^{Amigo} from the 1AL.1RS rye translocation (Olivera Firpo et al.,

2017). These genes are widely present in North American winter wheat (Olson et al., 2010; Yu et al., 2010; Zhang et al., 2014) and are among the few genes conferring TTKSK resistance in the United States (Jin & Singh, 2006). The 37 German wheat cultivars carrying *Sr24* and *Sr38* described in this study are susceptible to the *Pgt* race TKKTP.

In this study, we found four cultivars ('Hyland', 'Memory', 'Pilgrim PZO' and 'Tybalt') with seedling resistance to all tested isolates including TTKSK (Ug99, Table 5). One possibility is that these cultivars carry both *Sr24* and *Sr31* that, in combination, would be effective against all the races evaluated. However, none of these four cultivars possessed both genes. Either *Sr24* or *Sr31* were confirmed by molecular markers to be present in three of these cultivars (*Sr24* in 'Tybalt' and *Sr31* in 'Hyland' and 'Pilgrim PZO'), indicating the presence of additional uncharacterized effective gene(s) in these cultivars that could be used as sources of stem rust resistance in German and international breeding programmes. The resistance of cultivar 'Memory' could not be attributed to any single seedling resistance gene based on our data.

All *Pgt* races isolated from the 2013 epidemic in Germany were avirulent to *Sr31*, and only one race (TKKTP) was virulent to *Sr24* (Olivera Firpo et al., 2017). Because this race was not included in the field inoculations, the seedling and adult-plant tests showed agreement for all 16 cultivars possessing one of these two genes displaying low stem rust field scores from 1 to 4. All inoculated races were virulent to *Sr38* (Olivera Firpo et al., 2017), but the disease severity of 29 cultivars carrying this gene varied between 0.55% and 16.26%. In the study of Zhang et al. (2014), *Sr38* was the most effective gene in the field test in the USA with an average severity for lines carrying only *Sr38* of 5.2%. Nine cultivars with *Sr38*, a gene to which there was a high virulence frequency in the inoculated *Pgt* population, displayed strong resistance (0.55%–3.42% stem rust severity, Table 6). These cultivars might possess additional, yet unidentified *Sr* genes or even quantitative resistance. These hypotheses must be tested in further studies.

4.2 | Adult-plant resistance

Thirty-four (43%) winter wheat cultivars in our study had no detectable seedling resistance genes (Table 7). Their adult-plant responses

TABLE 7 Adult-plant stem rust severity (LS-means) in field evaluations in 2014–2016 and significance of differences between each entry and the most susceptible cultivar 'Julius' for winter wheat cultivars with no detectable seedling *Sr* genes

Cultivar	Adult-plant severity					Significance compared to Julius ($p < .01$) ^a
	2014 (%)	2015 (%)	2016 (%)	Mean (%)	Mean score (1–9)	
JB Asano	—	3.91	5.53	2.71	3	Yes
Orcas	6.92	2.51	—	6.22	4	Yes
Dekan	5.18	6.89	8.10	6.93	4	Yes
Discus	8.62	4.32	8.95	7.75	5	Yes
Matrix	6.59	13.60	6.95	9.19	5	Yes
Florian	8.06	8.73	—	9.60	5	Yes
Kometus	4.79	14.93	10.76	10.05	5	Yes
Naturastar	4.42	6.43	20.31	10.18	5	Yes
Butaro	2.97	5.12	24.87	10.59	5	Yes
Magnus	5.58	19.26	—	12.83	6	No
Apertus	6.42	20.36	14.78	13.56	6	No
Zeppelin	13.94	11.33	—	14.03	6	No
Akratos	5.32	18.76	20.08	14.24	6	No
Patras	12.25	14.01	16.06	14.29	6	No
Lear	9.08	28.26	8.97	15.28	6	No
Bussard	11.54	17.49	—	15.39	6	No
Inspiration	4.53	32.08	12.75	15.81	6	No
Kurt	7.80	24.62	—	16.43	6	No
KWS Ferrum	14.86	21.39	13.91	16.93	6	No
Waxydie	—	23.46	15.29	17.41	6	No
Johnny	—	24.79	18.46	19.47	6	No
Cubus	17.82	17.88	24.33	20.15	6	No
Edward	8.69	24.64	30.18	20.41	6	No
Gourmet	10.49	34.66	20.35	21.21	6	No
Attraktion	—	24.73	23.89	22.31	7	No
Apian	11.80	38.34	19.86	22.71	7	No
Kredo	9.50	43.08	20.76	23.54	7	No
Estivus	17.09	31.78	—	24.78	7	No
Boxer	9.08	42.40	—	24.95	7	No
KWS Smart	—	47.66	9.31	26.62	7	No
Toras	14.30	33.31	39.64	28.13	7	No
Tobak	16.50	42.65	28.11	28.36	7	No
Diantha	—	37.38	25.47	29.47	7	No
Julius	—	26.63	60.57	41.51	8	—

^aMultiple tests with SIMULATE after Edwards and Berry (1987).

varied from 2.71% to 41.51% stem rust severity. Interestingly, this group of cultivars did not provide the highest levels of resistance; however, nine cultivars were moderately resistant (score 3–5) at the adult-plant stage as confirmed by their statistically significant difference to the most susceptible check cultivar 'Julius.' These nine cultivars were susceptible to all tested *Pgt* races at the seedling stage. Because we included *Pgt* isolates in the field inoculations with all virulences compared to the international isolates except *Sr24* and *Sr31*, two genes that were not postulated in these cultivars, these results indicate the presence of APR. Monogenically inherited APR is

uncommon for stem rust (Pathan & Park, 2007) with only a few genes known (*Sr2*, *Sr55*, *Sr56*, *Sr57*, *Sr58*) to confer a measurable level of resistance (Singh et al., 2015). We analysed all field-tested 79 winter wheat cultivars by molecular markers for four of these five genes and found no cultivars with *Sr2* or *Sr55*. For *Sr57*, we detected only one cultivar with the resistance alleles (Waxydie) which was moderately susceptible in the field (17.41%, Table 7). The marker for *Sr58* was confirmed in 41 cultivars, but unfortunately, this marker is not diagnostic according to Gina Brown-Guedira, ARS-USDA, who developed the marker Gina Brown-Guedira,

(pers. commun.). Therefore, the genotypes of this group might possess quantitative resistance. This type of resistance is often growth-stage-specific, depends on environment, acts only partially and is inherited by many genes with small effects (Miedaner, 2016). Knott (1982) reported several additively acting genes mediating stem rust resistance, a model that perfectly fits with the concept of quantitative resistance (Geiger & Heun, 1989). Another possibility could be residual stem rust resistance conferred by a resistance gene effective to another rust (Pathan & Park, 2007). Molecular mapping studies of the three most resistant wheat cultivars of this group (JB Asano, Orcas and Dekan) would be worthwhile for enhanced understanding of this yet unknown APR to stem rust.

5 | CONCLUSIONS

These results indicate that most German varieties are being protected by very few resistance genes and many varieties lack resistance genes effective against emerging *Pgt* races. In the last 4 years, only 26% of the total German wheat-growing area was planted with stem rust resistant cultivars as indicated by combining results from the current study with the multiplication areas of the respective cultivars (Tables S1 and S2, BSL, 2016). Therefore, there is a need to incorporate other stem rust resistance genes in German wheat. The results of our study help in breeding for stem rust resistance as they indicate cultivars with APR that could be used as crossing parents.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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